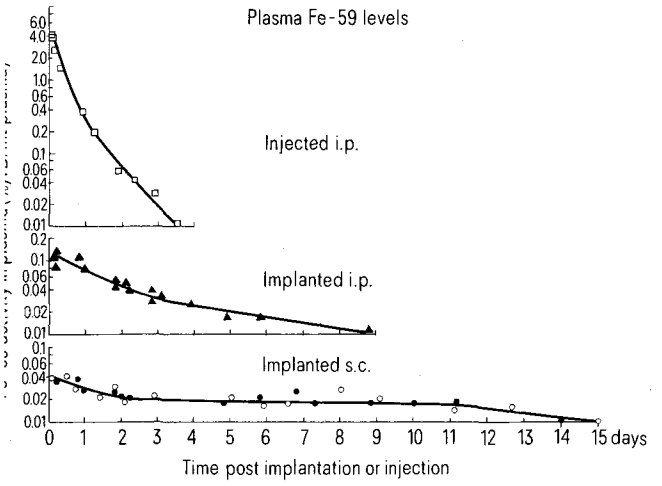


Fig. 1. Plasma levels of $^{59}Fe$ after injection and during infusion. Each point is an average of duplicate samples. The open (−○−) and closed (−●−) circles represent data from 2 different animals.

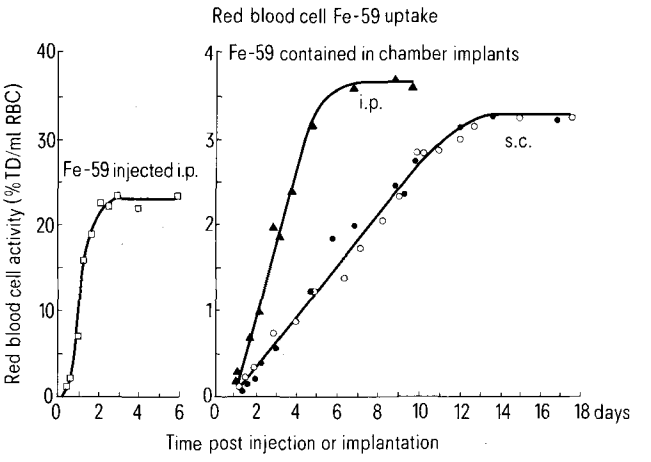


Fig. 2. Rate of uptake of $^{59}Fe$ into RBC after injection and during infusion. Each point is an average of duplicate samples. The open (−○−) and closed (−●−) circles represent data from 2 different animals.

constant, levels of isotope could be maintained between the 2nd and 12th day when infused from a s.c. implant. The fall-off seen thereafter may be due to a membranous covering which formed over the chamber[5]. Two-thirds of the initial $^{59}Fe$ still remained in the chamber at this time.

Figure 2 shows the kinetics of $^{59}Fe$ appearance in circulating RBC. After injection of isotope, the level of labeling increased linearly between 0.5 and 1.5 days. However, linear uptake of $^{59}Fe$ was sustained for 10 days when the isotope was infused from a chamber implanted SQ.

This method could have many applications. It should find use in the determination of steady-state kinetics. It provides an improvement over earlier methodologies[6] for studies on the dynamics of bone. It should make possible a more accurate determination of rates of exchange, accretion, and resorption of bone mineral.

The method should find application, as well, in pharmacodynamic studies. It could prove useful for the administration of highly toxic chemotherapeutic agents which need to be given in low doses over an extended period of time.

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## A simple technique for the measurement of $^{3}H$- and $^{14}C$-radioactivity per µg DNA in fixed tissue[1]

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*Summary.* A simple, rapid and versatile technique for scintillation counting of double labelled fixed tissue is described. Furthermore, DNA determination can be performed on the same tissue digest.

The uptake of labelled thymidine, measured by scintillation counting, has been used as a representation of the proliferative activity in tissue[2,3]. It is often preferable to express the radioactivity per µg DNA rather than tissue weight or protein. We have recently described a method for the fluorometric determination of DNA in tissue previously fixed in ethanol/acetic acid, a method which includes solubilisation of the tissue in warm NaOH followed by neutralization. In this report we demonstrate that scintillation counting can be performed simply and accurately in the same solution.

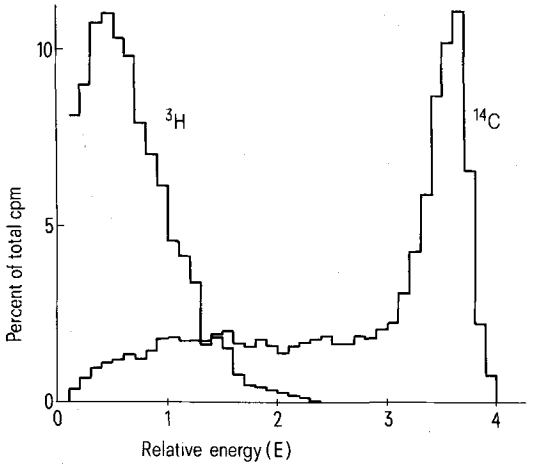
*Materials and methods.* The tissue used was rat small intestine, labelled in vivo with [methyl-$^{3}H$] thymidine (5 Ci/mmole) and [2-$^{14}C$] thymidine (57 mCi/mmole). It was fixed in abs.ethanol/glacial acetic acid 3:1 (v/v), and stored in 70% ethanol. Samples weighing 20–40 mg were incubated in 2.5 ml of 1 mole/l NaOH at 60 °C for 2–3 h until clear, and neutralized with isomolar HCl. This solution was also used for fluorometric determination of DNA with ethidium bromide, modified for fixed tissue[4]. For scintillation counting, 1 ml sample solute was added to 10

Counting efficiency recorded as percent recovery of internal standard. (Mean ± SD in 20 samples)

| Channel | $^{3}H$ | $^{14}C$ |
|---|---|---|
| Window | 0.3 ± 1.1 | 2.4 ± 2.2 |
| Efficiency $^{3}H$ | 22.5 ± 0.4 | < 0.1 |
| Efficiency $^{14}C$ | 36.9 ± 0.7 | 48.2 ± 0.7 |

ml Unisolve 294 (Kock-Light Laboratories Ltd., England) in polyethylene vials (W. Zinsser, Frankfurt, Germany) and counted in an automatic beta-gamma spectrometer (Nuclear Enterprise 8312) at room temperature.

As internal standards, 10 µl of [4-$^3$H] toluene (2.13 µCi/g) and [methyl-$^{14}$C] toluene (0.504 µCi/g) were used, giving 41,009 dpm and 9693 dpm, respectively. All radioactive isotopes were obtained from The Radiochemical Centre, Amersham, England. Spectra were recorded using a fixed window ($\Delta E = 0.1$), on 100 µl of standards added to 1 ml unlabelled tissue digest.




Energy spectra recorded for $^3$H and $^{14}$C in the emulsifying liquid scintillator Unisolve 294. Spectra were recorded in a Nuclear Enterprise 8312 automatic beta-gamma spectrometer, using a window, $\Delta E = 0.1$.

*Results.* Spectra obtained for $^3$H and $^{14}$C are shown in the figure. Tritium has a remarkably narrow range, being unrecognizible at $E > 2.4$, while $^{14}$C has a peak at $E = 3.6$ as well as a plateau at lower energies. Thus, by appropriate choice of windows, $^{14}$C can be counted without disturbance from $^3$H. In the following, the settings given in the table were used.

Miscibility with waterphase: 10 ml Unisolve 294 was found to accept 0–0.1 ml and 0.6–2.0 ml of water, and 0–0.1 and 0.8–2.0 ml 1 M NaCl or tissue solute. Between 0.1 and 0.6–0.8 ml, the mixture becomes cloudy. This is in accordance with the data given by the manufacturer.

Efficiency expressed as percent recovery of internal standard is given in the table.

Storage: Background was negligible even at immediate counting. In the tissue samples, counting efficiency declined linearly with time, reading 80% of the initial value after 5 days at room temperature.

*Discussion.* The quenching by this method was found remarkably stable and reproducible. Thus, for routine use, quenching correction should be unnecessary. It also gives an unusually good separation between $^3$H and $^{14}$C, which is further improved by using rather narrow channels. Finally, DNA can be measured in the same solute, which implies that weighing errors, which may be troublesome in tissue samples, are avoided.

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## A simple method for obtaining high plating efficiencies with cultured insect cells

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*Summary.* A technique has been developed for obtaining absolute plating efficiencies as high as 79% for cultured *Trichoplusia ni* (TN-368) cells. The method involves the use of conditioned medium and MES, BES, or ACES buffers. Cell growth and morphology are not altered under these conditions.

Methods of obtaining high plating efficiencies with cultured mammalian cells are well established[2,3]. Similar techniques for cultured insect cells are not as well defined. Cloning procedures have been developed for insect cells, but these are not useful in plating experiments[4,5]. McIntosh and Rechtoris have devised a method permitting colony formation in soft agar with a plating efficiency of 1%[6]. While this method may be effective for cloning cells, the cell morphology appears to be altered and the method is not readily useful for survival studies. Richard-Molard and Ohanessian have utilized the 'feeder layer' method[3] to successfully clone *Drosophila* cells[7]. In this paper a method is presented for obtaining high absolute plating efficiencies (up to 79%) with *Trichoplusia ni* (TN-368) cells in liquid medium. This method is potentially useful for evaluating the survival of insect cells following various stresses such as exposure to UV, X-rays, or chemicals. A series of cell growth curves (prepared by hemocytometer counts) are compared to a cell survival curve (obtained by colony formation) following X-irradiation of the cells in order to demonstrate the significance of the colony formation technique.

*Methods.* The TN-368 cell line, derived from the cabbage looper, *Trichoplusia ni*, was used in this study[8]. 5 different variations of TNM-FH medium[9] were tested: 1. Regular TNM-FH (pH of about 6.2), 2. conditioned TNM-FH

Plating efficiencies of *T. ni* (TN-368) cells in modifications of TNM-FH medium

| Medium | Plating efficiency (%) |
|---|---|
| | (n = 19)* |
| Regular TNM-FH | 19.6 ± 1.4** |
| | (n = 18) |
| Conditioned TNM-FH | 49.8 ± 2.3 |
| | (n = 22) |
| Conditioned TNM-FH with MES*** | 57.9 ± 1.8 |
| | (n = 23) |
| Conditioned TNM-FH with BES*** | 79.2 ± 2.0 |
| | (n = 17) |
| Conditioned TNM-FH with ACES*** | 65.1 ± 2.6 |

* Total number of dishes from 3 experiments; ** 1 SE.; *** Initial pH adjusted to 6.0–6.2 with 1 N KOH.